PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/675, 31/16

A1

(11) International Publication Number: **WO 99/13885**

(43) International Publication Date: 25 March 1999 (25.03.99)

(21) International Application Number: PCT/US98/19126

(22) International Filing Date: 16 September 1998 (16.09.98)

(30) Priority Data:
97/8319 16 September 1997 (16.09.97) ZA

(71) Applicant *(for all designated States except US)*: FRANGOLD HOLDINGS LIMITED [ZA/ZA]; Benstra Building, 6th Floor, 473B Church Street, Arcadia, Pretoria 0083 (ZA).

(71) Applicant *(for MG only)*: DAVIS, Joanne, T. [US/US]; 714A 15th Street, Arlington, VA 22202 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LANDAUER, Carl, John [ZA/ZA]; 124 Kate Street, Riviera, Pretoria 0084 (ZA). DU PREEZ, Gabriel, Johannes [ZA/ZA]; 322 Kerkenberg Avenue, Villiera, Pretoria 0186 (ZA). SCHILLACK, Volker, Reinhard [ZA/ZA]; 872 – 28th Avenue, Rietfontein, Pretoria 0084 (ZA). ATTFIELD, Derrick, Cecil [ZA/ZA]; 41 Frodo Crescent, Pierre von Rijneveld, Centurion 0157 (ZA). VAN DEN BOGAARDEN, Johannes, Beyers [ZA/ZA]; 76 Frans Oerder Street, Groenkloof, Pretoria 0181 (ZA).

(74) Agent: NATH, Gary, M.; Nath & Associates, Suite 750, 1835 K Street, N.W., Washington, DC 20006–1203 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MODULATION OF IMMUNE RESPONSES

(57) Abstract

A method of affecting or modulating for stimulation or suppression of the immune cell product pursuant to an immune response to an external or endogenous immune stimulant in an animal comprising the steps of administering to such animal a non–toxic immune modulating effective quantity of a compound selected from the group consisting of compounds of the general chemical formula (I) R_1–CO–NR_2R_3 wherein R_1 is selected from the group consisting of H and lower (i.e. C_1 to C_3) alkyl and C_2 to C_3 alkenyl groups; R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower (i.e. C_1 to C_3) alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another from a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof. The method is useful in the treatment of a variety of ailments associated with inappropriate immune responses and the invention provides for the use of compounds of Formula (I) in the manufacture of medicaments for use in the treatment of such ailments and for pharmaceutical preparations containing same.

BEST AVAILABLE COPY

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

MODULATION OF IMMUNE RESPONSES

FIELD OF THE INVENTION

THIS invention relates to the treatment of conditions in the human body which are associated with inappropriate immune responses. More particularly, this invention is concerned with the treatment of conditions in which the inappropriate immune response is associated with inappropriate-immune cell metabolic activity which, in turn, is mediated, or at least thought to be associated with the Tyrosine Kinase Cascade in which Protein Tyrosine Kinase, hereinafter referred to as "PTK", plays an important role, or with Cycline Dependent Kinase, hereinafter referred to as "CDK" in the immune cell. The invention consequently provides for the treatment of and for medicaments for use in the treatment of, a large variety of ailments associated with inappropriate immune responses in the animal body, which term is intended herein to include the human body.

BACKGROUND TO THE INVENTION

It is well known that immune cells play an important role in the immune system of the animal body. A variety of such cells have been identified. Amongst these the T lymphocytes, also known as T-cells, and the B-lymphocytes, also known as B-cells, have been recognized for their

important role in the immune response of the animal body against foreign infections. Additionally, other cells capable of producing immunological mediators have an immune function. The normal, or appropriate response of such cells to an extraneous stimulant, for example a viral or bacterial infection of the animal body or to endogenous immune stimulants, for example oncogenic transformation, is to cause the activation of the metabolic pathways of the cells in issue resulting in the production of immunological mediators including cytokines, lymphokines, chemokines, growth factors and cytotoxic cells and usually also gives rise to the proliferation of such immune cells. The immunological mediators, in turn, perform or complement the function of neutralizing the infective agent, or toxins released by it, as part of the natural infection combating or healing process of the body. These pathways involve complex biochemical and signalling systems to which further reference is made below.

It is also well-known that, for reasons which are not fully understood at present, the response of the immune system is sometimes abnormal and gives rise to the expression by, and secretion from, the immune cells of products considered to be inappropriate. While such abnormality may be associated with an abnormal proliferation of such cells, it is not necessarily the case. Such abnormality in immune cell activity may simply be manifested by the secretion of an inappropriate quantity of immune cell products without necessarily being associated with an increase in the

proliferative activity of such cells.

Inappropriate immune response has been implicated in a large variety of ailments affecting the human and animal body. The present invention is directed at the treatment of such conditions as will appear below. In the conditions in question the manifestation of an inappropriate immune response, including the presence of cytotoxic cells, and/or of an inappropriate quantity of the immune cell products required to combat a particular infection or condition, give rise to what may be thought of as an attack by the immune system on specific organs of the body itself, or even to a non-specific immunological attack on the body generally, rather than such attack being appropriately directed at the extraneous or endogenous antigen or agent or metabolites such as toxins produced thereby. The target organs of such inappropriate attack are diverse and, as will be seen from the list of conditions enunciated below, encompass the skin, the muscles, the skeletal structure, the joints, the blood, the brain and nervous system, the internal organs including the bowel, kidneys, lungs, liver, and even the sensory organs including the eyes.

In some of the conditions or syndromes with which this invention is concerned a primary or initiating causative agent or stimulant for the immune response has been identified. It is however a feature of such conditions or syndromes that the immune response may persist even after

the causative agent or stimulant, such as, for example, a viral or microbial infection or other form of antigen, has been eliminated by the body's own defense mechanism, namely the immune system, or by means of pharmaceutical intervention, or by a combination thereof. In other cases no, or at least no as yet identified causative agents are known. All these conditions are considered to be immune mediated ailments.

For a clear understanding of the nature and impact of the present invention it is necessary briefly to explain the current understanding of the nature of immune response activity in the body. Immune cells, and in particular T lymphocytes are dependent for their biological function on signal transduction through the T cell receptor which is unique to, and present only on T lymphocyte surfaces. The T cell receptor is a complex group of surface molecules including CD_4 or CD_8 surface molecules. The CD_4, CD_8 and certain other surface molecules are capable of transmitting a signal from the external surface of the cell membrane to the internal environment of the cell i.e. to the cytoplasma of the cell. These signals are transmitted through an enzyme pathway known as the Tyrosine Kinase Cascade. Activated Tyrosine Kinases phosphorilate certain proteins leading to the development of new signal intermediates. In this cascade the enzyme known as Protein Tyrosine Kinase [PTK] plays an important role. This enzyme is a protein featuring free sulfhydryl groups. The Tyrosine Kinase cascade ultimately terminates in the expression of specific genes in the T cell. These genes

respectively code for a variety of immunological mediators including cytokines and for structural proteins required for cell proliferation. The immune response mediated ailments with which this invention is concerned are, as mentioned before, associated with uncontrolled expression of immunological mediators by the immune cells and uncontrolled cytotoxic cell activity.

DISCUSSION OF PRIOR ART

Dimethylformamide or DMF is a volatile, non-ionic liquid at room temperature and is well known for its use as an industrial solvent. It is used, amongst others, in the manufacturing process of various products for human consumption, including in the manufacturing processes of pharmaceutical products. Its toxicity is well studied. It is known to be non-toxic at levels many times higher than the levels with which the present invention is concerned. Such toxicity as DMF has at high levels of concentration in the human body is attributed to its depletion of glutathione in hepatocytes in the liver resulting in hepatocyte necrosis.

It has been claimed by Cryopreservation Technologies CC in PCT application PCT/US96/19697 published on 26 June 1997 under WO 97/22248 that dimethylformamide and related compounds may be used in the treatment of viral and/or microbial infections. That publication cites

examples showing that DMF may be administered transdermally to a patient to obtain a DMF in blood level aimed to be 100 ppm, but the levels reported as actually achieved varied from this target, in HIV positive patients. It further reports that after such treatment with DMF for a period of less than three weeks, the viral load in the patient had dropped from 120 000 to 500/ml. It also reports an improvement in the symptoms of acne and German measles in patients treated with DMF to achieve a DMF/blood level of 50 - 100 ppm. That disclosure relates to the use of DMF as an anti-viral and anti-microbial agent and is silent on the immune response regulatory properties of the compounds with which this invention is concerned, and on the use of such compounds in the treatment of immune cell related illnesses or the use of such compounds in the manufacture of medicaments for use in the treatment of such conditions.

OBJECT OF THE INVENTION

It is an object of the invention to provide a method of modulating an immune response in the animal or human body and of treating ailments associated with inappropriate immune responses in the animal, including human, body.

DESCRIPTION OF THE INVENTION

It has now surprisingly been found, in contradistinction with the prior art knowledge regarding the toxicity and anti-viral and anti-bacterial properties of DMF that such product may be used in sub-toxic dosages to modulate the immune responsive metabolic processes in cells having an immunological function by affecting such processes to stimulate or suppress the expression or secretion of immunological mediators by such cells without destruction of the cells through the administration of DMF to the body.

According to the present invention there is thus provided a method of affecting an immune response in an animal comprising the steps of administering to such animal a non-toxic immune modulating effective quantity of a compound selected from the group consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group

wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof.

The immune response to be affected by the above method may be an immune response of the immune cells forming part of the immune system of the body.

The immune cells may be T lymphocytes and or B lymphocytes.

The method may be performed to reduce the expression or secretion of immune cell products in the body.

According to a further aspect of the present invention there is provided a method of treatment of an animal afflicted with an ailment associated with inappropriate immune responses in that animal, comprising the steps of administering to such animal a non-toxic therapeutically effective quantity of a compound selected from the group consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the

group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof.

According to another aspect of the invention it relates to use of a compound selected from the group consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof

in the manufacture of a medicament for use in a method of treatment of an animal afflicted with an ailment associated with inappropriate immune responses in that animal.

Further according to the present invention it relates to a pharmaceutical composition comprising a compound selected from the group consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof

in a pharmaceutically acceptable dosage form for use in the treatment of an animal afflicted with an ailment associated with inappropriate immune responses in that animal.

In all the treatment related aspects of the invention the ailment to be treated may be any one of the following:

Systemic Lupus Erythematosus [SLE]

Scleroderma [Systemic sclerosis]

Vasculitis Syndrome [including Wagener's thrombosis and all forms of Giant cell arthritis]

Dermatomiositis

Asthma

Adult Respiratory Distress Syndrome [ARDS]

Systemic Inflammatory Response Syndrome [SIRS]

Inflammatory Bowel Disease

Chronic Hepatitis

Rheumatoid Arthritis

Rheumatic fever

Myasthenia Gravis

Multiple Sclerosis

Psoriasis

Eczema

Multiple myeloma

Reiter's Syndrome

Glomerulonephritis

Polymyalgia Rheumatica

Ankylosing spondylitis

Polyarteritis Nodosa

Allergic Rhinitis

Diabetes mellitus

Optical Neuritis

Acute Transversmielitis

Head Injuries

Spinal Cord injuries

Post sub-Arachnoidal Bleeding Vasospasma

In all the aforementioned aspects of the invention the compounds of choice is dimethylformamide and its metabolites namely N-methylformamide, N-methyl-isocyanite and its carbamates, and N-acetyl-S-(N-methylcarbamoyl)cysteine and any prodrugs of such metabolites.

Further according to the invention the method is performed by administering to the patient to be treated a quantity of DMF sufficient to maintain a DMF-plasma level of between 0.001% and 0.1% and most preferably between 0.01% and 0.05%. Likewise, the medicament according to the invention is adapted in use to administer to the patient a quantity of DMF at such rate as to achieve and maintain a DMF-plasma level of between 0.001 % and 0.1% and most preferably between 0.01% and 0.05%.

Also, in all aspects of the invention the compound may be administered by any route of administration, such as orally, nasally, rectally, intravenously, intramuscularly, subcutaneously or transdermally. The preferred route of administration of the compound is transdermally. Preferably, however, it is administered by a transdermal patch.

As will appear from the examples below, mixed lymphocytes were exposed

to a non-specific stimulator of lymphocytes, such as PHA at 1 to 10 μg per 200 μL. When these lymphocytes were concurrently also exposed to very low concentrations of DMF a decreased metabolic activity was demonstrated as evidenced by the fact that these cells were less capable of reducing a patented [U.S. Patent No. 5,501,959] redox indicator. This inhibition of metabolic activity is not the result of direct cell toxicity as is evidenced by the fact that the therapeutic concentration to effect inhibition of metabolic activity and therefor proliferation, is lower than a concentration which causes activation of cell metabolism and therefor proliferation.

By bringing the immune active cells in need of modulation into contact with DMF or any of its metabolites in a therapeutic concentration, it is possible to diminish the metabolic activity of cells with immune function. By decreasing the metabolic activity of T lymphocytes the responsiveness of these T lymphocytes to certain T lymphocyte specific antigens and antigen, major histocompatibility complex molecule combinations will be severely inhibited. It is the antigen specific inhibition of T lymphocyte responses that forms the basis of the clinical application of DMF to modulate immune cell mediated diseases proposed by the present invention.

For the transdermal administration of DMF to a patient in need of treatment according to the invention it is proposed to use a multi-purpose transdermal administration system that is able to deliver a variety of drugs

including DMF and the above-mentioned related compounds therapeutically.

The patch design parameters considered important include the following:

1. The patch must have pre-determined dimensions as it determines the amount of active ingredient [drug] absorbed over a certain time.

2. Drug administration must not be time dependent.

3. Drug concentration should be variable according to patient profile.

4. The patch must be stable, and deliver repeatable therapeutic concentrations of agent [drug] that is administered.

5. The tempo of drug [agent] administration is determined by the skin, thus the desorption of the drug is through the membrane should be the same or very close to the absorption tempo of the skin.

6. The patch and the drug must have a relative long shelf life.

To achieve the above requirements a patch has been designed with the following features:

1. High density nylon backing material.

2. Safety border consisting of nylon of PATFE [Teflon].

3. Low density septa NYLON or PTFE.

4. Hydrophilic or hydrophobic NYLON or PTFE membrane [Depending on which drug is administered].

5. Membrane pore size of 0.05-0.45 micron depending on which drug is administered.

6. Diatomaceous earth [SiO_2] adsorbent material.

7. Stabilisation agent [Salting agent to decrease evaporation as well as homogenic weight distribution through the membrane].

8. Suitable skin adhesive which is not reactive with the drug.

9. Anti-irritation agent - Vitamin E [This agent can be applied before, during or after application of the agent to protect the skin against side effects].

More particularly a patch was made up as follows:

1. Backing material and septa

Description	Round semi-transparent nylon disk 0.1-0.4 mm thick
Diameter	20 - 100 mm
Average mass	100 mg - 600 mg
Septa description	Round soft polypropylene/polyethylene with nylon or PTFE backing
Septa diameter	5 - 25 mm
Septa thickness	0.2 - 1 mm

2. Teflon membrane

Description	Round white membrane
Diameter	20 - 100 mm
Average Mass	50 - 500 mg
Pore Size	0.2 - 0.8 micron

3. Teflon border ring

Description	Round semi-transparent 0.1 - 0.4 mm thick
Inner diameter	10 - 90 mm
Outer diameter	20 - 100 mm

Average mass	20 - 200 mg

4. Absorbent material

Description	Silicon dioxide [diatomaceous earth]
Mass / patch	1 - 10 g
Stabilisation agent description	Sodium chloride and magnesium / calcium carbonate

APPLICATION TECHNIQUE

Indirect administration of the drug such as DMF may be done by introducing a known amount of the active agent with a syringe into the Silicon dioxide adsorbent after the patch had been applied to the skin.

The following advantages are obtained by using this technique:

1. Controlled agent administration from an ampoule/container onto a patch.

2. Therapeutic agent dosage can be predetermined according to patient's profile.

3. By making the agent concentration dependent, the time of treatment and area of skin exposure stays constant during treatment.

4. Patch and agent can be applied with confidence without overdosing the patient.

5. Patch and agent are easy administered and the patient can be discharged after administration.

6. Patch is very stable, has an unlimited shelf life, and agent administered from an ampoule has at least a two year expiry date, unless otherwise stipulated.

EXAMPLES OF THE INVENTION

The invention will now be demonstrated with reference to the following examples without thereby limiting the scope of the invention to the illustrative embodiments.

EXAMPLE 1

Peripheral human lymphocytes were isolated from whole blood of healthy volunteers by using a density gradient separation technique well described

and known in the immunology research literature. Briefly described, by this technique peripheral whole blood is diluted 1:2 with phosphate buffered saline or cell culture media such as RPMI1640. A density gradient such as Histopaque 1077 [Sigma Cat # 1077] is then layered underneath the diluted peripheral blood, taking care to create a sharp interface. The density gradient is then centrifuged at 600 g for 20 to 25 minutes. After centrifugation a clear buffy coat layer containing mostly lymphocytes is easily visible. This layer is then removed and further processed by placing the buffy coat in an additional tube and washing the cells washed 3 times in PBS or cell culture media. After each of the wash steps the cells are centrifuged at 400 g for 9 minutes. After the third wash the lymphocytes are collected from the pellet. The cells are counted and diluted to the desired cell concentration.

A variety of DMF concentrations were prepared using complete cell culture medium as the diluent. The following concentrations were prepared: 1%, 0.1%, 0.05%, 0.01% and 0.001%.

The isolated lymphocytes were diluted to the required concentration using a complete cell culture medium.

The complete cell culture medium used in the above steps contained RPMI1640 with HEPES 20-25 mM, L-glutamine 1 mM, dimercapto-ethanol

$2x10^{-5}$ mM and 10% heat inactivated fetal calf serum.

The prepared lymphocytes were placed in 96 well cell culture plates at a concentration of 150,000 cells per well. PHA [Phyto-Heame-Agglutinin] was added to each test well at a concentration of 1 to 10 μg per 200 μL. At the same time DMF was added to each of the test wells at the predetermined concentration and the culture wells were incubated at 37°C in 5% CO_2, 95% O_2 atmosphere for 1 to 4 days.

A redox indicator sold under the trademark AlamarBlue was added to each of the culture wells at 10% volume:volume. The cultures were incubated for 18 hours with AlamarBlue prior to determining the absorbance at 570nm with 630nm as a reference.

The results may be summarised as follows:

The culture in which peripheral lymphocytes from healthy volunteers were exposed to PHA and DMF at 1% concentration for 24 hours reduced 102% of the quantity of AlamarBlue reduced by the culture in the normal PHA growth well. However, for the cultures in which DMF was added at a concentration of only 0.05% reduced only 52% of the quantity of AlamarBlue reduced by the culture containing PHA stimulated lymphocytes. This demonstrates a significant inhibition of metabolic

activity in the culture well which correlates very closely with the level of cell proliferation in the particular well.

When comparing the metabolic activity as indicated by AlamarBlue reduction of unstimulated lymphocytes to unstimulated lymphocytes exposed to low concentration of DMF, a 40% decrease in reduction of AlamarBlue is observed for lymphocytes exposed to 0.05% DMF concentration. [See Figures 1 and 2 which represent typical results of a number of repetitions of the experiment]

EXAMPLE 2

To demonstrate the postulated mechanism of action of the DMF in causing depression of lymphocyte metabolic activity a further series of experiment was performed. In these experiments the lymphocytes of healthy volunteers were isolated using the technique described in Example 1. After the lymphocytes were isolated the lymphocytes were exposed to DMF at various concentrations for varying lengths of incubation time. The DMF concentrations used were 0.1%, 0.01%, and 0.001% and the incubation times were: 5, 15, 30, 75, 120 and 180 minutes. The Tyrosine kinase activity was determined using a commercially available Tyrosine kinase assay kit available from Boehringer Mannheim [Catalogue Number 1534505]. The results were as presented in the graphs of Figures 3 and 4. Over a 3 hour

exposure to a 0.01% concentration of DMF the tyrosine kinase activity of the tested lymphocytes were inhibited by 71%. It is pointed out that in this graph the lower the absorbance reading, the higher the degree of tyrosine kinase activity inhibition. The general shape of the graphs remained the same regardless of the time allowed for development of the colour reaction. The results support the postulate on which the present invention is based that DMF at certain concentrations, when exposed to lymphocytes for a certain period of time serves to block Tyrosine kinase activity. There is the possibility that the observed decreased activity in tyrosine kinases in the tested lymphocytes might be the result of stimulation of certain tyrosine phosphatases. The presented graphs are typical examples of many graphs obtained.

EXAMPLE 3

The PTK inhibitory activity of DMF and two of its metabolites was further demonstrated by comparing it to a commercially available PTK inhibitor Piceattanol marketed by Boehringer Mannheim under #1534505 on two cells lines named HELA, a Cervix cancer cell line and HEP3B a liver cancer cell line. The following results were obtained:

The inhibitor was used in 10% DMSO solution.

The test was carried under the following conditions and yielded the results shown below:

Slank: 0,043
Funct: Cntrl

Endogenous phosphatase: 20.3%
Endogenous phosphorylation: 0.14 [0,208]

		Activity	Av. Inhibition
HELA			
Inhibitor : [Pilleattanol + 10% DMSO]	0,076 [0,132]	66% 75%	29,5%
Clean Cells	0,115 [0,176]	100%	0%
2% Metabolite I	0,099 [0,167]	86% 95%	9,5%
0,2% Metabolite II	0,074 [0,140]	64% 80%	28,5%
2% Metabolite II	0,055 [0,091]	48% 55%	48,5%
0,2% DMF	0,073 [0,146]	63% 83%	27%
2% DMF	0,042 [0,082]	37% 47%	58%
HEP3B			
Inhibitor : [Pilleattanol + 10% DMSO]	0,055 [0,111]	60% 60%	40%
		66% 75%	29,5%
Clean Cells	0,091 [0,186]	100%	0%
2% Metabolite I	0,090 [0,150]	99% 81%	10%
0,2% Metabolite II	0,036 [0,060]	40% 32%	64%
2% Metabolite II	0,095 [0,095]	60% 51%	44,5%
0,2% DMF	0,080 [0,123]	88% 66%	33,3%
2% DMF	0,040 [0,064]	44% 34%	61%

IC50 1,7 - 17μM was established

IC50 1,7 - 17μM was established

CODE

Metabolite I	-	N-methylformamide
Metabolite II	-	N-methylisocyanite
DMF	-	Dimethylformamide

CLAIMS:

1. A method of affecting an immune response in an animal comprising the steps of administering to such animal a non-toxic, immune modulating effective quantity of a compound selected from the group of compounds consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof.

2. The method of claim 1 wherein the immune response to be affected by the method is an immune response of the immune cells forming part of the immune system of the body.

3. The method of claim 2 wherein the cells are selected from the group consisting of T lymphocytes and B lymphocytes.

4. The method of claim 1 performed to reduce the expression and secretion of immune cell products in the body.

5. A method of treatment of an animal afflicted with an ailment associated with inappropriate immune responses in that animal, comprising the steps of administering to such animal a non-toxic therapeutically effective quantity of a compound selected from the group consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof.

6. The use of a compound selected from the group consisting of compounds of the general chemical formula (I)

$$R_1\text{-}CO\text{-}NR_2R_3 \qquad (I)$$

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof

in the manufacture of a medicament for use in a method of treatment of an animal afflicted with an ailment associated with inappropriate immune responses in that animal.

7. A pharmaceutical composition for use in the treatment of an animal afflicted with an ailment associated with inappropriate immune responses in that animal comprising a compound selected from the group consisting of compounds of the general chemical formula (I)

R_1-CO-NR_2R_3 (I)

wherein

R_1 is selected from the group consisting of H and lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups;

R_2 and R_3 are the same or different and each is selected from the group consisting of H, lower [i.e. C_1 to C_3] alkyl and C_2 to C_3 alkenyl groups which may optionally be halogenated or hydroxylated, or which may in combination with one another form a $-(CH_2)_n-$ group wherein n is any number from 2 to 5, or the group $-(CH_2)_2-O-(CH_2)_2$ and metabolites and prodrugs thereof in a pharmaceutically acceptable dosage form.

8. The method of claim 5, the use of claim 6 or the composition of claim 7 wherein the ailment is selected from the group consisting of:

Systemic Lupus Erythematosus [SLE]

Scleroderma [Systemic sclerosis]

Vasculitis Syndrome [including Wagener's thrombosis and all forms of Giant cell arthritis]

Dermatomiositis

Asthma

Adult Respiratory Distress Syndrome [ARDS]

Systemic Inflammatory Response Syndrome [SIRS]

Inflammatory Bowel Disease

Chronic Hepatitis

Rheumatoid Arthritis

Rheumatic fever

Myasthenia Gravis

Multiple Sclerosis

Psoriasis

Eczema

Multiple myeloma

Reiter's Syndrome

Glomerulonephritis

Polymyalgia Rheumatica

Ankylosing spondylitis

Polyarteritis Nodosa

Allergic Rhinitis

Diabetes mellitus

Optical Neuritis

Acute Transversmielitis

Head Injuries

Spinal Cord injuries

Post sub-Arachnoidal Bleeding Vasospasma.

9. The method, use or composition of any one of claims 1 to 8 wherein the compound is dimethylformamide.

10. The method of claim 9 wherein the dimethylformamide is administered to the patient to be treated in a quantity and at a rate sufficient to maintain a DMF-plasma level of between 0.001% and 0.1%.

11. The method, use or composition of claim 9 or the method of claim 10 wherein the medicament is, or is adapted to be administered transdermally.

12. The method, use or composition of claim 11 wherein the DMF is administered, or is adapted to be administered by means of a transdermal patch substantially as herein described.

FIG. 1

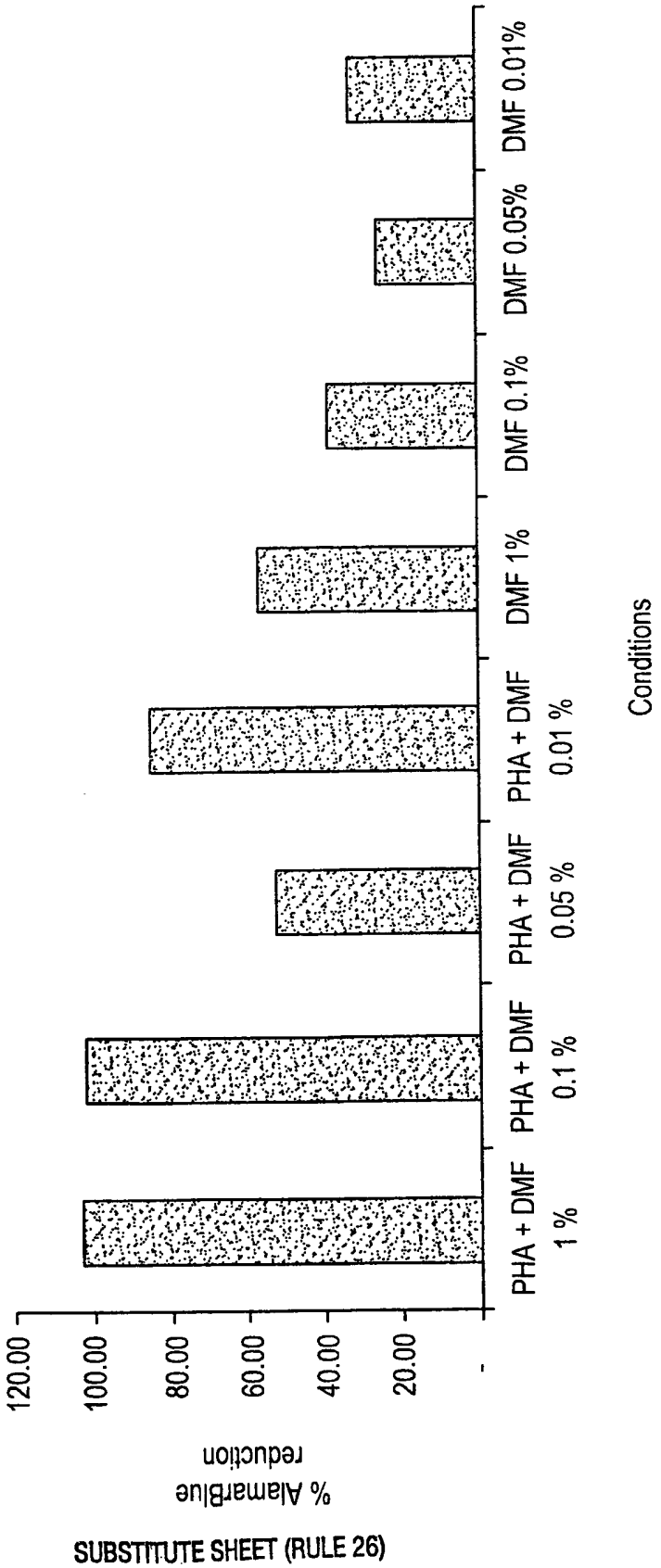

120.00
100.00
80.00
60.00
40.00
20.00
-
% AlamarBlue reduction
PHA + DMF 1 %
PHA + DMF 0.1 %
PHA + DMF 0.05 %
PHA + DMF 0.01 %
DMF 1%
DMF 0.1%
DMF 0.05%
DMF 0.01%
Conditions

FIG. 2

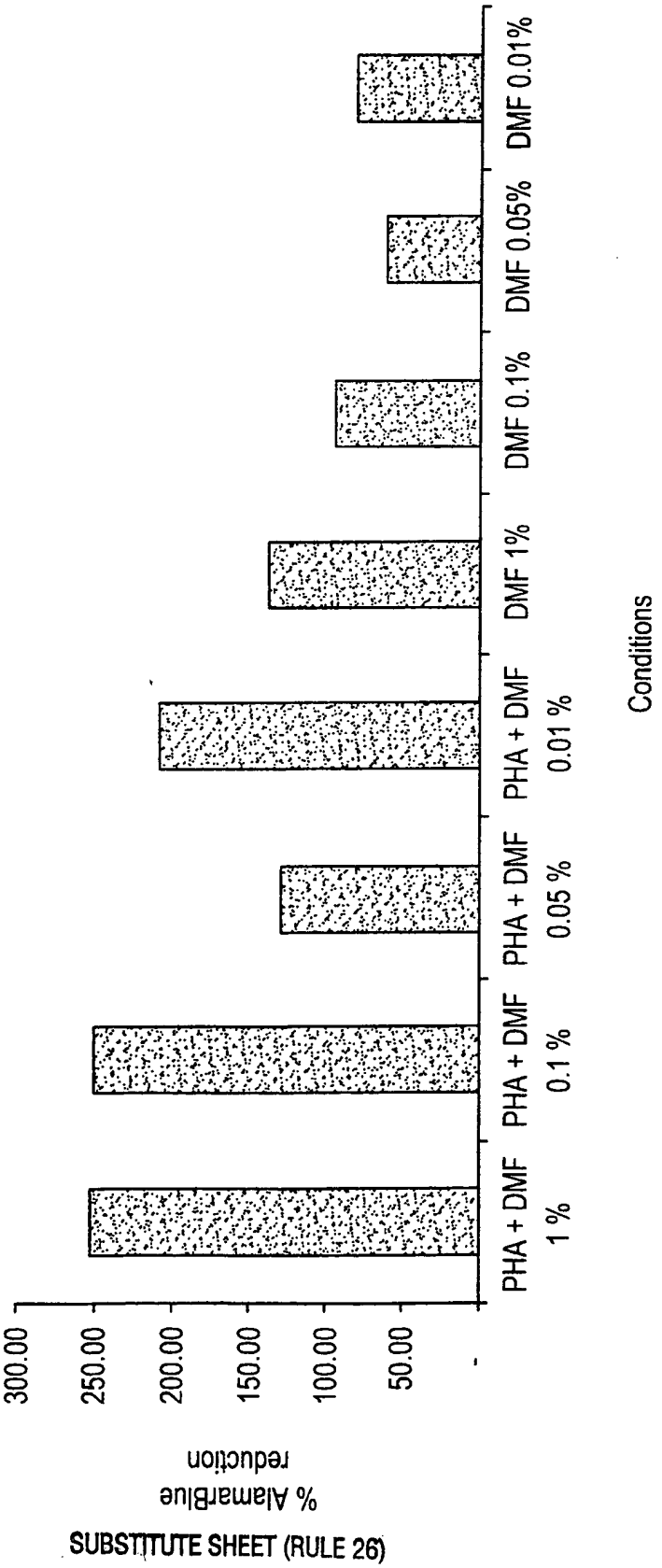

300.00
250.00
200.00
150.00
100.00
50.00
-
% AlamarBlue reduction
PHA + DMF 1 %
PHA + DMF 0.1 %
PHA + DMF 0.05 %
PHA + DMF 0.01 %
DMF 1%
DMF 0.1%
DMF 0.05%
DMF 0.01%
Conditions

FIG. 3

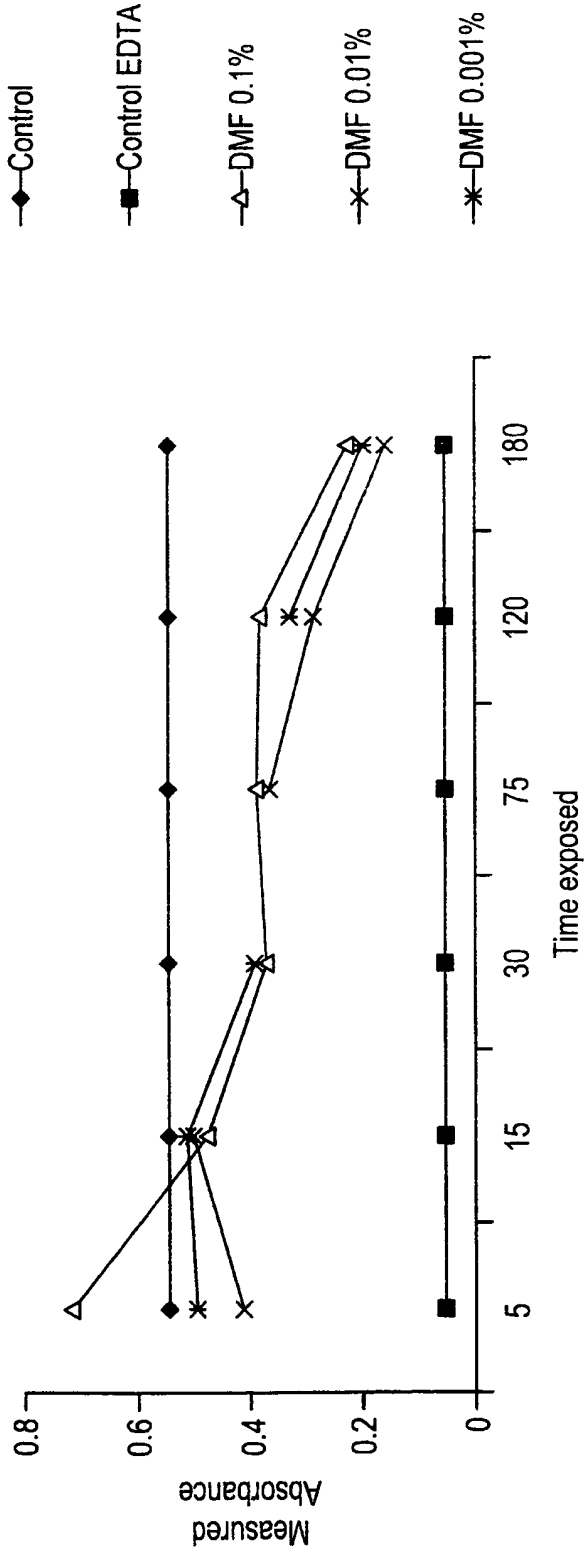
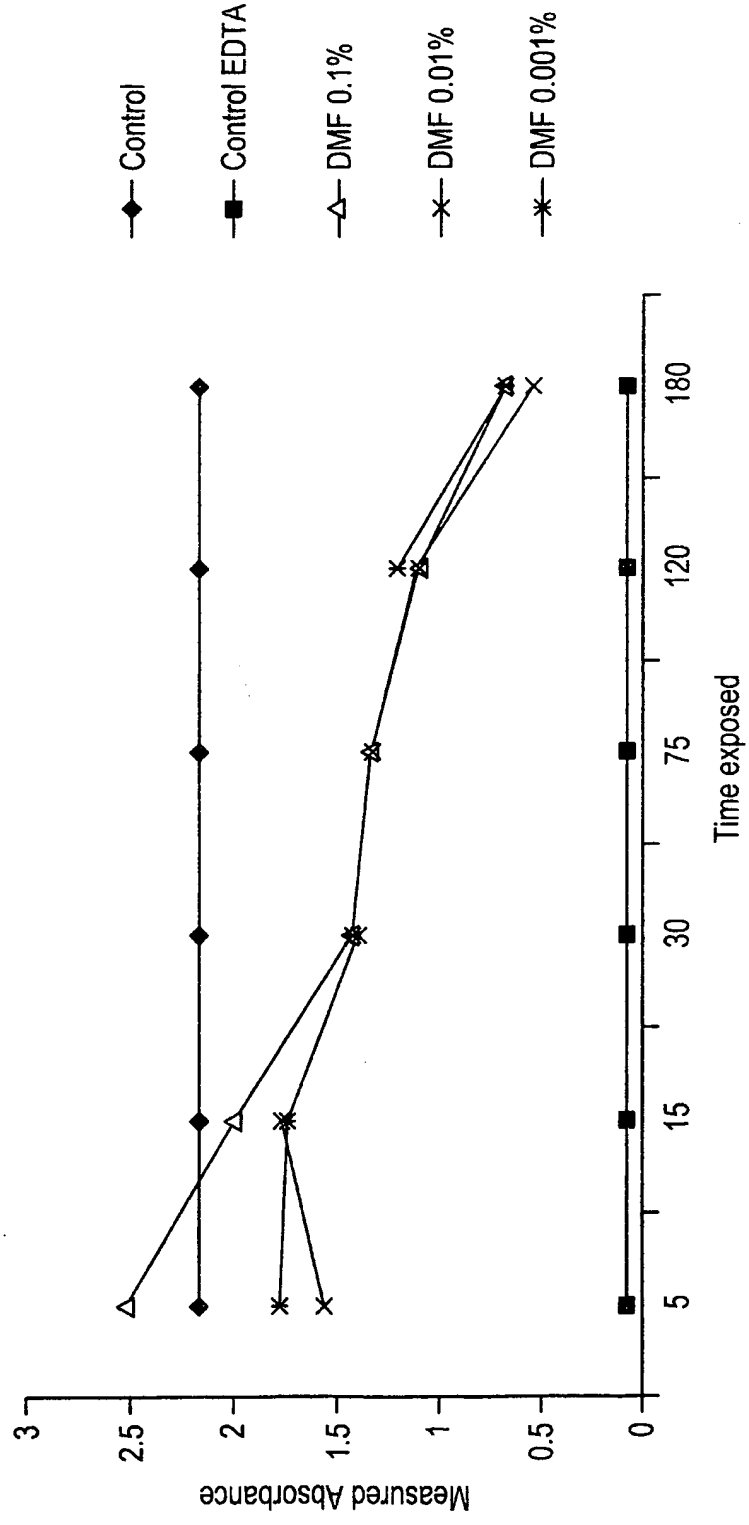

Control
Control EDTA
DMF 0.1%
DMF 0.01%
DMF 0.001%
Measured Absorbance
0.8
0.6
0.4
0.2
0
5
15
30
75
120
180
Time exposed

FIG. 4


Control
Control EDTA
DMF 0.1%
DMF 0.01%
DMF 0.001%
Measured Absorbance
3
2.5
2
1.5
1
0.5
0
Time exposed
5
15
30
75
120
180

BEST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/19126

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/675, 31/16
US CL :514/627, 629

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/627, 629

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN COMPOUNDS AND ANTIVIRAL METHODS OF USE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chemical Abstracts, Vol. 73, No. 23, issued 07 December 1970, ARTINI et al. "Amides and amines, their synthesis and antiinflamatory and analgesic activity", pages 345-346, columns 2 and 1, abstract no. 120262r, Arzneim. -Forsch., 1970, Vol. 20(8), pages 1009-18, see entire abstract.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
24 NOVEMBER 1998	23 DEC 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RUSSELL TRAVERS Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★